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Molecular epidemiology of rabies virus in Nepal

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Background: Rabies is endemic and priority zoonotic disease in Nepal. Death of 150 people and 200 animals has been reported annually in this country however molecular epidemiology of rabies has not been well understood. A study was performed by analyzing 10 rabid animal's brain samples (7 dogs, 1 goat, 1 buffalo and 1 alpaca) collected in Nepal from 2011–2012 to know the circulating clade of Rabies virus in Nepal.

Methods & Materials: All 10 samples were analyzed in Changchun Veterinary Research Institute, OIE reference laboratory in China in 2012, by performing molecular characterization and phylogenetic analysis. First of all, Rabies virus or antigen was detected in 10 samples by Fluorescent Antibody Test and Mouse Inoculation Test. Viral RNA was extracted from only 8 positive samples by using QIAGENTM RNeasy Kit. Amplification of N and G genes of rabies viral RNA was performed by using primers with Invitrogen Super Script III First-Strand Synthesis System and the Platinum Taq DNA Polymerase High Fidelity kit (Invitrogen, USA). PCR products were analyzed by Agarose gel electrophoresis (Biorad molecular biology grade agarose). Analysis of sequence was made by using Clustal W of MEGA 5.1 and DNASTar v. 7.2 (DNASTAR Inc., USA). Alignment analysis was performed with reference sequences from Nepal and surrounding countries, such as, China, India, Pakistan and Sri Lanka.

Results: All the data demonstrate that viruses isolated in Nepal belong to the classic rabies virus. N gene phylogenetic analysis of Nepalese sequences with reference sequences showed that Arctic related subclades AL-1 and AL-3 are circulating in Nepal. One buffalo brain was AL-1, and 7 dogs brain were AL-3. Further analysis indicates that these Nepalese isolates were genetically close to viruses isolated in India.

Conclusion: The identification of isolates in this study has contributed to our understanding of the natural circulation and transmission of rabies viruses between Nepal and India. However, Himalayan Mountain provides a natural barrier and probably constrains the spread of rabies between Nepal and China. Our findings suggest that national geographical boundaries and border controls act as effective barriers to halt the spread of rabies from Nepal into adjacent regions.

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Typhoid fever surveillance in africa program (TSAP): Constructing a geospatial sampling frame for random sampling of households

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Background: Household sampling is used for cost-efficient, in-depth population-based investigations, including the assessment of healthcare utilization. Probability sampling requires a comprehensive sampling frame (household list), which did not exist at sites of the Typhoid Fever Surveillance in Africa Program (TSAP) in Pikine/Senegal, Pietermaritzburg/South Africa, and Wad Medani/Sudan. Here we describe the methodology of constructing a geospatial sampling frame.

Methods & Materials: Sites were diverse in population size, population density, area size, and administratively-identified sub-areas. They varied in topography, vegetation, and composition of formal/informal settlements and single-/multi-story structures. We used high-resolution GoogleEarthPro[®] satellite imageries for manually enumerating (pinpointing) structures to construct a sampling frame at each site. Hand-held satellite maps and global positioning system (GPS) devices were utilized by interviewers for rapidly, precisely locating structures (households); structures were selected randomly and weighted proportional to the population size of each subarea (weighted-stratified sampling). The methodology was evaluated for accuracy by calculating ranges and quartiles of distances between GPS coordinates of original (as per sampling frame) and enrolled structures.

Results: A total of 46510, 63008, and 32794 structures were enumerated at sites in Senegal, South Africa, and Sudan, respectively. The fabrication of satellite maps, including the selection and pictorialization of structures, took approximately two to three weeks by site. We found that 87% (517/592), 56% (1,098/1,963) and 86% (471/549) of distances were <2.5 meters at the site in Senegal, South Africa, and Sudan, respectively. Calculations revealed furthermore that 59% of distances in rural, 57% in semi-urban and 50% in urban subareas of South Africa were <2.5 meters.

Conclusion: We have demonstrated that satellite imageries are a simple, precise instrument for creating a sampling frame at certain TSAP-sites. Hand-held satellite maps and GPS devices

allowed for the rapid, accurate location of selected structures. Some limitations remain. Correctly locating structures can be challenging for clustered, interlaced structures since interfering factors (obstructive/reflective structures, multi-story buildings, environmental diversity) may impact on the precision of GPS readings. Geospatial frames require constant updating, but could provide an approach for population-based investigations at probability samples of households in settings that are uncensused and lack longitudinal recording of socio-demographic and vital statistics.

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Genetic variability of the G-L intergenic region sequences of Indian rabies virus strains circulating in animals

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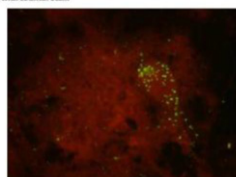
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Background: The evolutionary studies of rabies virus (RABV) have targeted the N or G gene, or recently the G-L intergenic region sequences. Among these, G-L intergenic region is considered as the most variable since it is not subjected to immunological selective pressure. The present study was undertaken to understand the genetic variability of RABV in animals in India based on the G-L intergenic region

Methods & Materials: Twenty seven brain samples from suspected rabid animals (22 dogs and five cattle) resourced from Karnataka (n=9), Kerala (n=5), Rajasthan (n=3), Tamil Nadu (n=2), Manipur (n=4) and Uttar Pradesh, Gujarat, Puducherry and Jammu Kashmir (n=1 each) were confirmed by Direct Fluorescent Antibody (DFA) assay (Fig.1). The samples were further subjected to Reverse Transcription Polymerase Chain Reaction (RT-PCR) along with Dr. Larghi's strain (PV-3462) as reference to amplify the G-L intergenic region. The PCR products (1354 bp) were purified, sequenced and compared to the corresponding sequences of RABV from different countries, CVS and PV strains obtained from GenBank. Phylogenetic tree was constructed using the nucleotide sequences corresponding to 423 bp of the 1354 bp amplicon. The branching pattern of the trees was constructed by the Neighbor Joining method using Mega 5 software version 5.02.

Fig. 1.: Rabid brain impression stained with the rabies virus anti nucleocapsid IgG-FFITC conjugate (Rabies DFA kit, Light Diagnostics, Cat# 05005) with counter stain



Results: The phylogenetic analysis revealed two major groups of RABV in India (Fig.2 & 3): Group 1 circulating all over India and Group 2 restricted to two Southern states, Tamil Nadu and Kerala. Group 2 RABV showed high homology with the Sri Lankan isolates. Group 1 was further sub-grouped into four, designated as 1a, 1b, 1c and 1d. Group 1a included the majority of isolates from Karnataka and Puducherry, and one from Kerala. Group 1b included RABVs from Rajasthan, Gujarat, Uttar Pradesh and Karnataka, whereas Group 1c included an isolate from Jammu & Kashmir along with isolates from Pakistan. Group 1d included isolates from Manipur and Bangladesh.

Fig. 2: Neighbour-joining phylogenetic tree of 27 RABVs and Dr. Larghi (PV-3462) reference RABV nucleotide sequences based on G-L intergenic region rooted with the CVS strains.

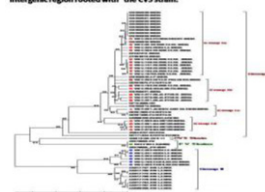
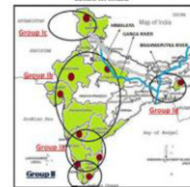


Fig 3: G-L intergenic region-based clustering of RABV from dogs and cattle in India



Conclusion: Rabies viruses circulating in animals in India belong to Genotype 1, and are genetically diverse. In the present study, the sub grouping of RABVs could be due to major geo-physical barriers such as Himalayan range, Western ghats and major rivers including Ganga and Brahmaputra.

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Prevalence of rickettsial infections in acute coronary syndromes in Sri Lanka: A case control study

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Background: Interest in the relationship between infection and atherosclerosis induced coronary heart disease has recently increased. Rickettsiae are a group of obligate intracellular pathogens who invade endothelial cells and cause vasculopathy. In a longitudinal nation wide study conducted in Taiwan, the incidence of acute coronary syndromes (ACS) in patients with scrub typhus was found to be higher than a comparison cohort (3.10 vs 1.92 per 1000 person-years). A 37% increased risk in subse-

